ADJUSTMENT OF CALORIC INTAKE BY THE KANGAROO RAT (*DIPODOMYS ORDII RICHARDSONI*) BECAUSE OF ENVIRONMENTAL TEMPERATURE CHANGE

By

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CHAPTER I

INTRODUCTION

Selection of Subject

Animals must select dietary items that provide sufficient calories to survive in their natural habitat. This study was performed to determine whether compensation of caloric intake is one of the adjustments of Dipodomys ordii richardsoni to variations in temperature. Since the kangaroo rat inhabits an area characterized by wide and often sudden temperature variations (U. S. D. A., 1964-1968) (Table I), and since changing environmental temperatures modify the caloric requirements of mammals, the animal should be an excellent subject for studying the adjustment of caloric intake to sudden variations in environmental temperatures. The relationship between temperature and caloric intake has not been documented for this species.

TABLE I

CLIMATOLOGICAL DATA FOR WOODWARD FIELD STATION DURING THE PREVIOUS 5-YEAR PERIOD (U. S. D. A. 1964-1968)

Year	Temperature Range in ^{o}F	Total Precipitation in Inches Per Year
1964	2 to 110	21.75
1965	5 to 102	19.25
1966	-6 to 104	17.29
1967	4 to 104	23.92
1968	0 to 104	20.11

Habitat of Subjects

Dipodomys ordii richardsoni inhabits the southern Great Plains where its preferred habitat is composed of sand dunes stabilized to different degrees by vegetation. The sand sagebrush (Artemisia filifolia) and tall and short grasses are the predominant vegetation. A detailed study of the habitat of this rodent species is that of McCulloch (1959).

Adaptation to Temperature

A species of animal must have evolved to meet the ecological requirements of its environment. A species that inhabits areas of extreme temperature variation must possess mechanisms of adaptation and adjustment. Ecological equivalents of the kangaroo rats, which

have evolved independently, inhabit all continents in which deserts or semi-deserts occur (Table II). These species have survived because of many morphological, physiological and behavioral mechanisms. Many studies on the water metabolism of the kangaroo rat and its ecological equivalents illustrate the mechanisms of adjustment to temperature by regulation of fluid intakes and outputs (MacMillen and Lee, 1969; Boice and Boice, 1968; Koford, 1968; Schmidt-Nielson, 1951, 1964). Little has been done, however, on the regulation of food intake as a mechanism of adjustment to temperature change.

Temperature and Dietary Changes

An animal adjusts to changing temperatures by regulating food intake (Howard, 1951). At different temperatures, an animal requires different caloric intake levels to maintain body temperature. The increase in intake of the ration with decreasing temperature, and the corresponding decrease with increasing temperature is well documented. The relationship between temperature and dietary changes has been demonstrated by Hatfield (1940) with Microtus, by Chenoweth (1917) and Eskridge (1958) with Peromyscus and by numerous investigators with laboratory rats (Dugal, 1945; Young, 1948).

Most dietary studies have dealt with laboratory rats, mice or other animals that through domestication have been inbred for docility and other non-adaptive traits in nature (Robinson, 1965; Lockard, 1968). Thus, wild species are often better able to adjust to physiological requirements than are domestic or laboratory animals (Kare, 1966).

Significant dietary experiments involving recently captured animals were performed by Eskridge (1958). He found that adding peanut

TABLE II

CLASSIFICATION AND LOCATION OF HETEROMYID-LIKE RODENTS FOUND IN DESERT OR SEMI-DESERT ENVIRONMENT*

Family	Genus	Common Name	Continent
Heteromyidae	Dipodomys	Kangaroo rat	North America
Dipodidae	10 genera	Jerboas	Africa, Asia
Muridae	Notomys	Australian Hopping Mice	Australia
Cricetidae	Phyllotis	Peruvian desert mice	South America
Cricetidae	Psammomys	Fat sand rat, Diurnal sand rat	Africa, Asia
Cricetidae	13 genera in subfamily: Gerbillanae	Gerbils	Africa, Asia

*Condensed from Walker et. al 1968.

butter to the diet of Peromycus increased survival time at freezing temperatures. Peanut butter, while certainly a source of fats, is also rich in protein. The role of fat in survival was evaluated with high and low fat diets. He found that at 32 F, 92% survived on the high fat diet; whereas, only 8% survived on the low fat ration. Although these experiments demonstrated the survival value of a high fat diet, they did not involve food selection. Therefore, he performed a third experiment in which there was a choice between the two diets and found that fat consumption was higher at lower temperatures ($P < .01$).

In Eskridge's experiments, as in most others on the relation of fat intake and temperature using mashes, the amount of fat or carbohydrate was varied. All the other constitutients, including protein, remained the same. The experimental design allowed selection of increased fat but not protein. Animals selectively increase both fat and protein intakes when given a choice. Dugal (1945) placed all dietary constitutients in separate containers and found that rats preadapted to diet, but not temperature, were able to survive -4 C only if they increased both casein and fat intake.

Caloric Adjustment

Selection

Warkentin, Warkentin, and Ivy (1943) demonstrated that selective feeding from purified foodstuffs occurred in laboratory rats. The rats maintained a relatively fixed caloric intake by alternate intake of fat and carbohydrate. Each foodstuff was presented in separate

containers to study food selection. The data collected was converted into grams per 100 grams of body weight, so that animals of different weights could be compared. About 95% of the rats made selections from the diet that were adequate for maintenance and growth. These selections resulted in a daily caloric intake from the diet that remained approximately the same for 192 days. The pattern of protein intake was generally the same as that for calories from the diet, but at a lower level. The caloric intake of carboyhdrates and fat was inversely related so that the calories from carbohydrate and fat when added to the calories from protein closely approximated the average intake of calories from the diet.

Volume

Adolph (1947) determined the effect of volume or other factors on caloric intake. Dried fortified milk was fed at concentrations from 0 to 30% solids in tap water. The volume ingested was adjusted to obtain the same amount of nutrients. This occurred down to the dilution of 2.6% solids at which point the rats ingested more than their body weight per day. Adolph also fed white rats food mixed with kaolin or cellulose as roughage. In both cases, the maximum roughage eaten was one-fourteenth of the body weight per day. The ingestion of roughage was not increased if the rats were previously deprived of food. The experiments suggested that the rats attempted to ingest an adequate volume to obtain sufficient calories. They lost weight because they were unable to consume the total volume necessary to maintain weight.

Rate and Precision

Janowitz and Hollander (1955) studied the precision and rate of caloric adjustment in dogs. There was a control period before intragastric feedings to determine the mean daily oral caloric intake and a control period after the intragastric feedings to determine if the animals returned to the same level of oral caloric intake. The volume of the intragastric feedings (50%, 100%, or 175% of the mean daily oral caloric intake) was adjusted to that eaten by each animal by adding water and was given hours before the oral presentation of food. After a period of adjustment to intragastric feedings, caloric intake was readjusted until calories from oral intake, together with calories from intragastric feedings closely approximated the calories taken orally during the pre-test control period. After intragastric feeding was stopped and equilibrium was reached, the oral intake was nearly the same as that during the pre-test control period. The above demonstrated the precision of caloric adjustment. The readjustment of intake began immediately upon the initiation or cessation of gastric feeding, but took many weeks to complete. Janowitz and Hollander concluded that adjustment of caloric intakes occurred with precision and that an extended period of time is required for complete adjustment to occur.

CHAPTER II

MATERIALS AND METHODS

Apparatus and Foodstuffs

Each kangaroo rat was individually housed in a 21.25 x 27.5 x 30 cm wire mesh cage which was provided with cotton for nesting material and a sand dish for dust bathing. A 250-ml Wahmann drinker was suspended outside the cage. A cone-shaped food cup filled with mash and a Wahmann food cup filled with Wesson Oil was placed on an aluminum foil apron at the front of the cage. The contents of the Wahmann food cup were overlaid with mesh netting (12 cm x 10 cm) to facilitate the removal of extraneous matter from the oil. The above arrangement allowed the sand to fall through the bottom of the cage, but food to be retained when spillage occurred. This arrangement also reduced the amount of sand spilled into the food containers and drinker.

The cages were placed in a cage rack in a 20' x 10' Percival environmental chamber in which the temperature and relative humidity were controlled (Table III). The animals were exposed to a 12-hour light, 12-hour dark cycle and to approximately 50% relative humidity. The chamber was maintained at approximately 30 C, 22.5 C or 15 C to study the subjects at either a raised or a lowered environmental temperature. The above predetermined values were used because they

provided equal physical increments of temperature change within the range of thermal neutrality of Dipodomys (Schmidt-Nielson, 1950).

TABLE III

MEANS FOR TEMPERATURE (C) AND RELATIVE HUMIDITY

EXPERIMENT	DAY	TEMPERATURE	RELATIVE HUMIDITY
Experiment I	1-20	22.3	48.4
	21-40	29.6	54.4
Experiment II	1-20	23.0	57.5
	21-40	16.6	63.4

The mash contained all essential dietary constitutients (Table IV). The quantity of each dietary essential required by this subspecies was defined earlier by a self-selection test using data on kangaroo rats taken during the final fifteen days of a 60-day test period (Milner and Harriman 1969).

TABLE IV

DIET DEFINED BY SELF-SELECTION

Constituents	Brand	Percentage of Diet	Caloric Value in kcal/g of Constituents	Caloric Value in kcal/ 100g of Mash
Sucrose	C and H Sugar	58.45	3.87	226.20
Casein	Skidmore	28.60	4.27	122.12
Salt Mix	U.S.P. XIV	2.00	0.00	0.00
Soybean and Cottonseed Oil	Wesson Oil	10.95	8.84	96.80

"Vitamin-free" soybean casein was the source of protein and contained all the essential amino acids. Sucrose was the only source of carbohydrate, and the U. S. P. XIV salt mix contained all necessary minerals. The soybean and cotton seed oil provided all essential fatty acids. The ratios of the components in the mash fed to the experimental animals are shown in Table IV.

Wesson oil had the highest caloric content of any of the food items. It was provided as 10.95% of the diet, but supplemental oil was provided in a separate container as an additional source of calories as well.

The vitamin solution was composed of 0.12 ml of Deca-Vi-Sol liquid vitamin concentrate per 100 ml of distilled water. This concentration was used because previously a study has shown that kangaroo rats

maintain normal weight over a 60-day period on this concentration (Milner and Harriman, 1969).

Procedure

The subjects were 31 kangaroo rats (Dipodomys ordii richardsoni) which had been captured on the Southern Great Plains Experimental Range on April 25, 1969. For the period before the study began, the animals were housed individually and were fed Purina Lab Chow and distilled water. After acclimation to the laboratory conditions the subjects were divided into 2 test groups. At the beginning of each test the subjects in that group were given wild bird seed (Kaytee) for six days before they were introduced to the experimental mash. The test animals were then allowed ten days to adjust to the mash before supplemental oil was presented. Oil was fed along with the mash for the following 40 test days. For the first 20 test days each group was maintained at 22.5 C. At the beginning of the second 20 days the temperature was raised to 30 C for the group in Experiment I and lowered to 15°C for the group in Experiment II (Fig. 1).

At 24-hour intervals records were made of individual body weights and of the consumption of the vitamin solution, mash, and oil. Although laboratory rats regulate food intake by calories rather than volume (Adolph, 1947; Warkentin, Warkentin and Ivy, 1943), a quantitative relationship between water intake and food consumption has been demonstrated in laboratory mice (Bing, 1931). Therefore, the volume of vitamin solution consumed was recorded, even though it obviously had no caloric value. Temperature and relative humidity were recorded daily throughout the experiment to verify the setting of the environmental

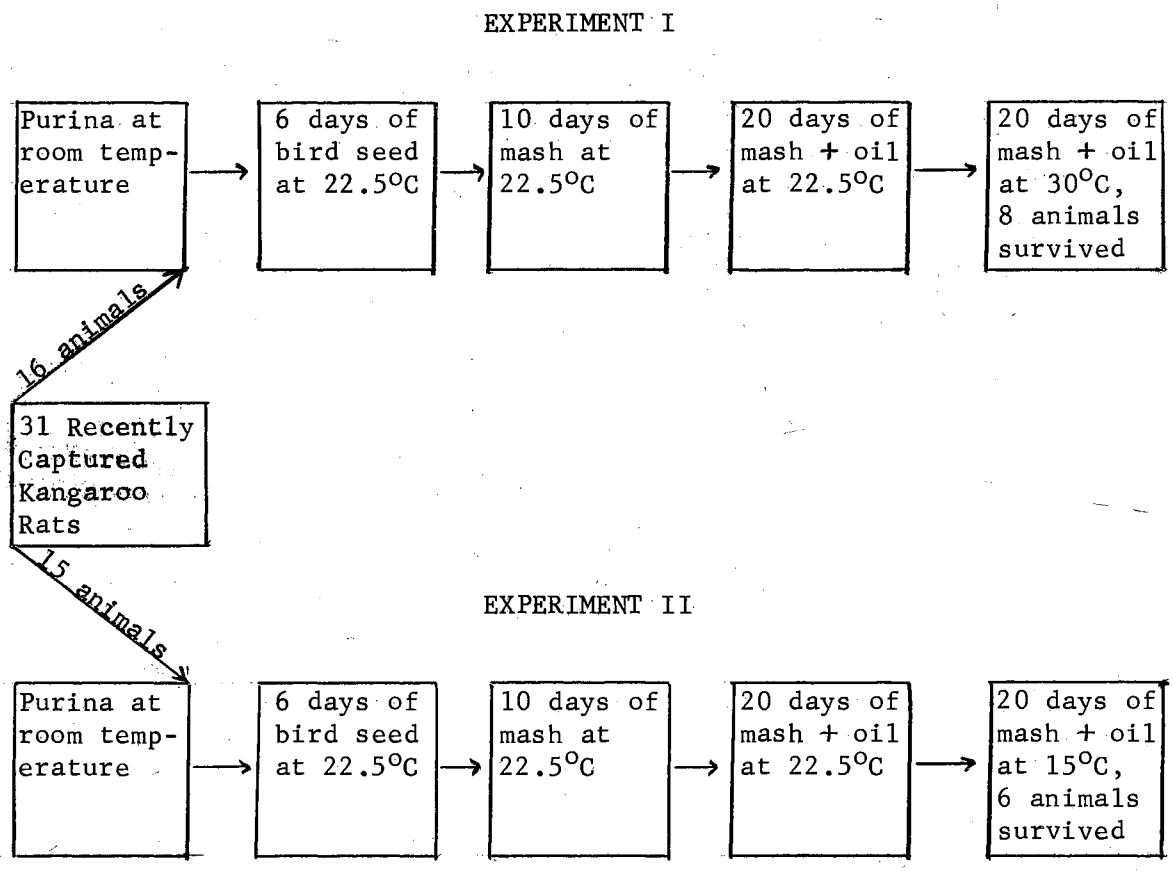

Figure 1. Time Sequences of Temperature Changes and Dietary Presentations

chamber. A control water drinker and food cup filled with Wesson oil were placed on both the highest and the lowest levels of the cage rack to allow correction for position effects on evaporation. Raw data were recorded as grams of mash or oil or as ml of vitamin-water consumed per 24 hour period.

Statistical Analysis

This experiment was analyzed as a split-plot in time. The main plot was a randomized plot consisting of two temperatures. Subjects were considered as blocks and days of each treatment were considered as a sub-plot. It should be noted, however, that each subject was used in both main plot treatments. Since there were only two treatments, the analysis of main plot treatment effect is valid. The analysis of the sub-plots may be questioned, since there were more than two sub-plots for each main plot treatment.

The data from Experiment I and Experiment II were analyzed separately due to the large difference in time between the running of the two experiments.

Only the data from the kangaroo rats surviving until the end of the test period were analyzed. Eight animals survived in Experiment I and six survived in Experiment II. Since survival indicated adjustment to the experimental diet, data from the non-surviving subjects were discarded.

Food or water records were corrected for spillage or evaporation. The consumed or corrected amounts were converted into caloric intake in calories per 100 grams of body weight for both oil and mash and into ml per 100 grams of body weight for the vitamin solution. This

was necessary because of the considerable discrepancies in weight among the test subjects. The body weights were also analyzed statistically.

CHAPTER III

RESULTS

Analysis of Variance

Main Plot

In Experiment I, temperature effects were observed on responses for oil and water at the 5% level. It should be noted that when the calories consumed from the mash and from the oil were combined, the temperature effect was no longer present. Temperature appeared to have no effect on body weight or mash intake. The analysis of variance for the responce variables from Experiment I were shown in Appendix A. The results are essentially the same in Experiment II, as in Experiment I, except that there was no temperature effect on water consumption and that at the 1% level there was an effect on both the oil and mash consumptions in Experiment II (Appendix B).

Sub-plot

Day effects were observed for mash and oil consumption and body weight in Experiment I. Day temperature interaction was observed for mash and diet consumption as well as for body weight. In Experiment II, day effects were noticed only for oil and water and day temperature interaction occurred only for water intake.

Results From Variables Measured

Caloric Intake

The caloric intake from the diet remained about the same when the environmental temperature was 30 C as when it was 22.5 C (Fig. 2). When the temperature was lowered to 15 C, the caloric intake from the diet was of the same magnitude as the intake at 22.5 C (Fig. 3).

The calories from the mash ingested at environmental temperature of 22.5 C was slightly lower and not the same pattern as at the increased temperature (Fig. 4). When the temperature was decreased to 15 C, the mash intake was distinctly higher than at 22.5 C (Fig. 5).

The calories ingested from the oil were distinctly higher at the raised temperature (Fig. 6) and distinctly lower at the lower environmental temperature (Fig. 7).

There was an inverse relationship between the calories from the mash and the calories from the oil in Experiment I (Fig. 8). This relationship was even more prominant in Experiment II (Fig. 9).

Vitamin-Water Intake

When the temperature was raised from 22.5 C to 30 C, which was within the range of thermal neutrality, there was a distinct, clearly defined, increase in water intake (Fig. 10). However, when the temperature was changed in the opposite direction, no change was evident in the ml/100g of body weight ingested (Fig. 11).

Body Weight

In Experiment I, body weight appears to be decreasing for the

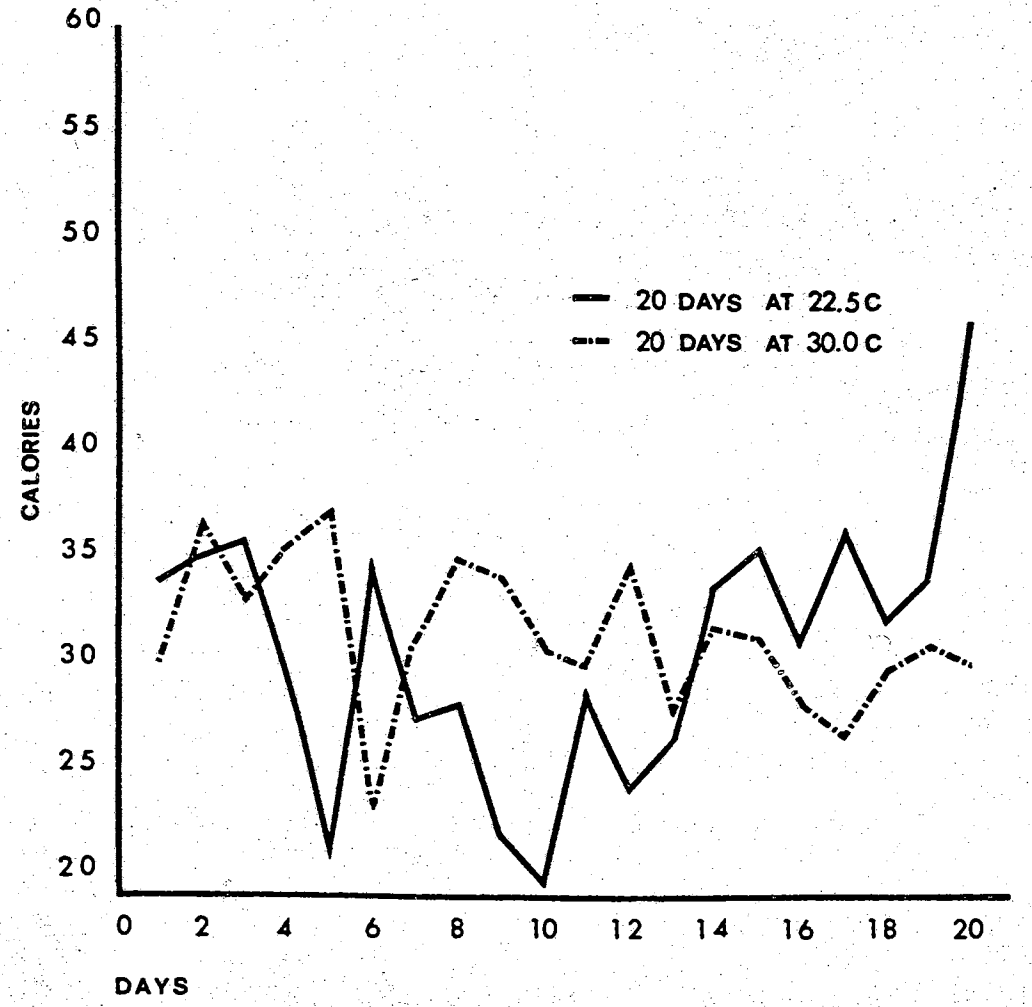

Fig. 2. Mean Daily Caloric Intakes from Diet in Calories/100g Body Weight for 8 Kangaroo Rats in Experiment I

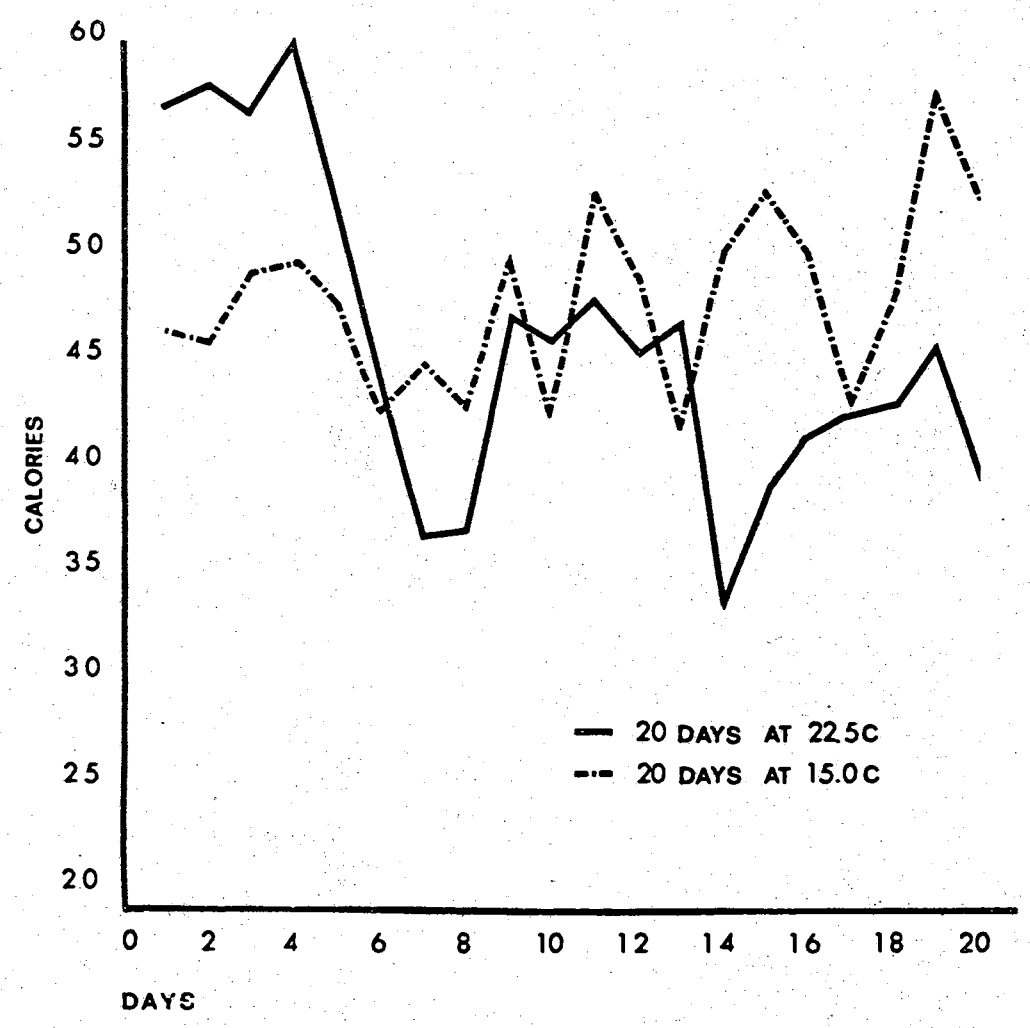

Fig. 3. Mean Daily Caloric Intake from Diet in Calories/ 100g Body Weight for 6 Kangaroo Rats in Experiment II.

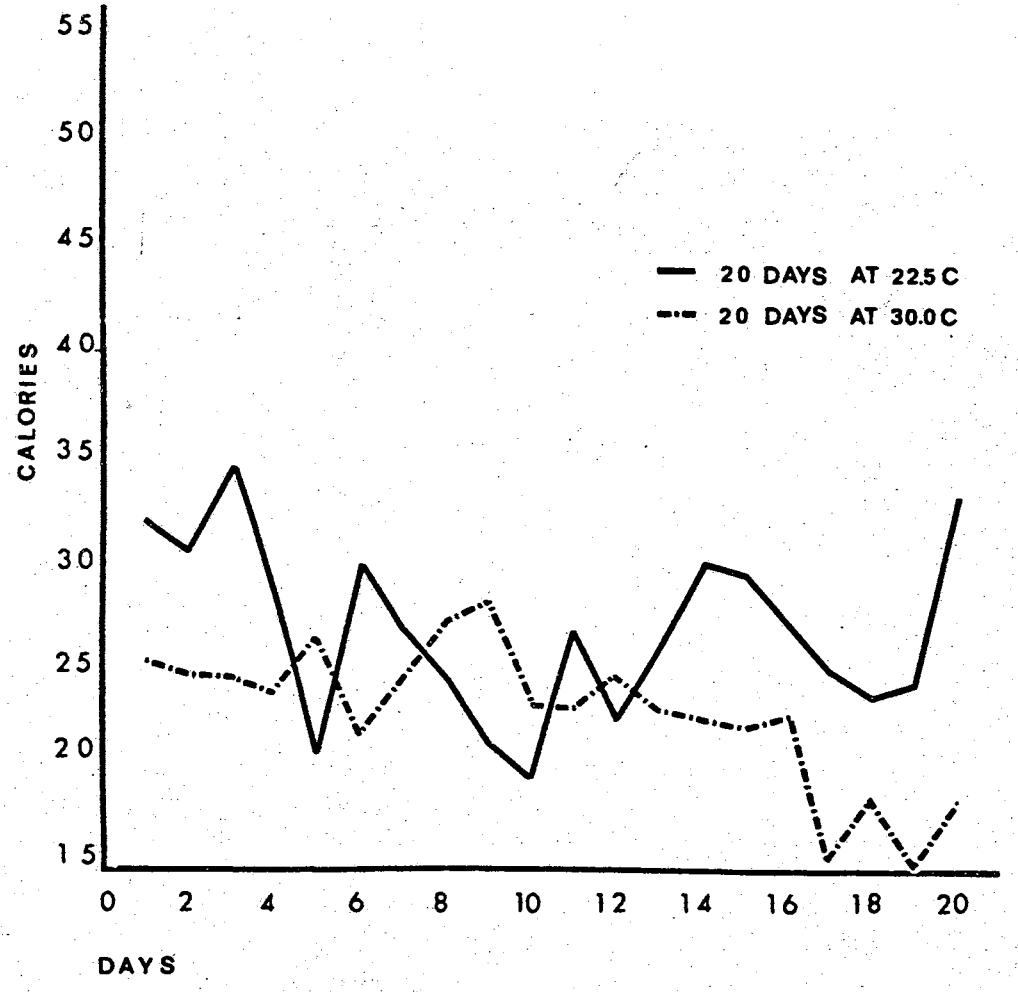

Fig. 4. Mean Daily Caloric Intakes from Mash in Calories/100g Body Weight for 8 Kangaroo Rats in Experiment I

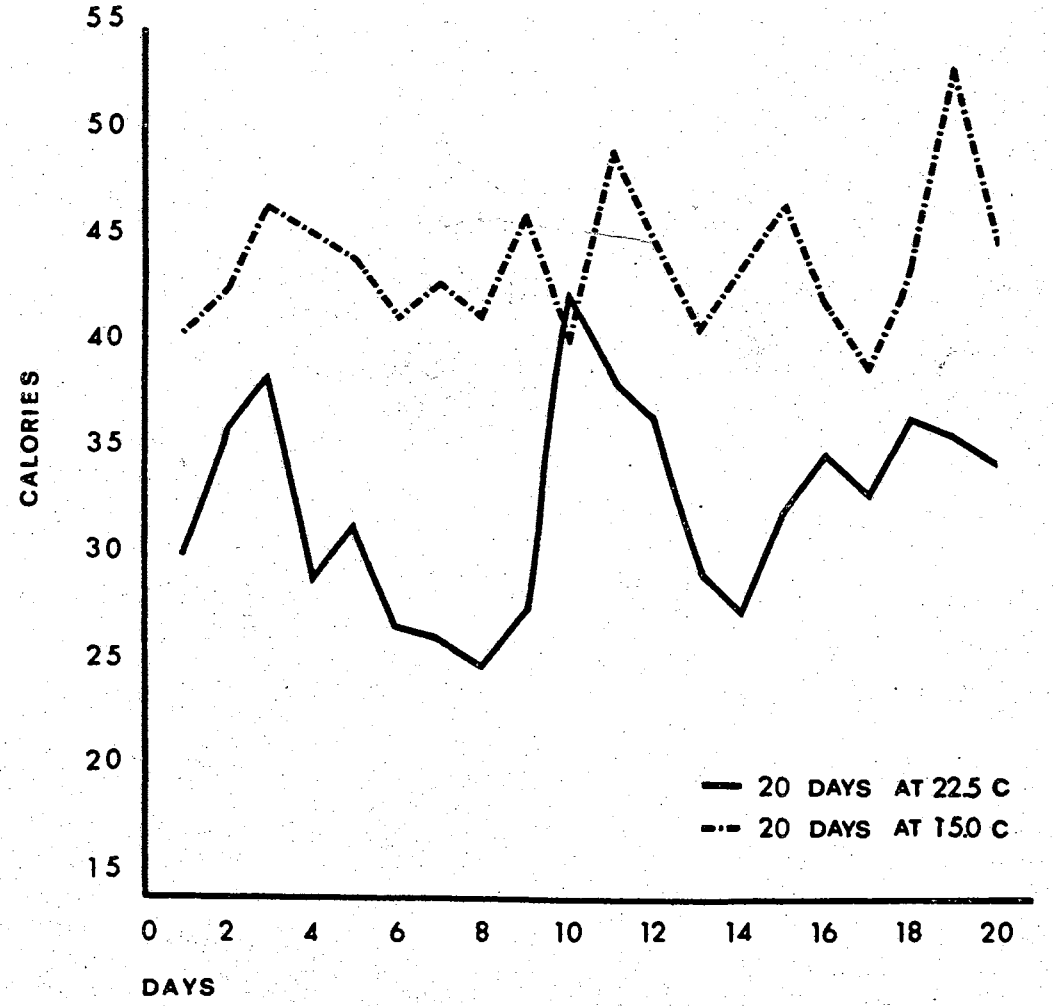

Fig. 5. Mean Daily Caloric Intake from Mash in Calories/ 100g Body Weights for 6 Kangaroo Rats in Experiment I

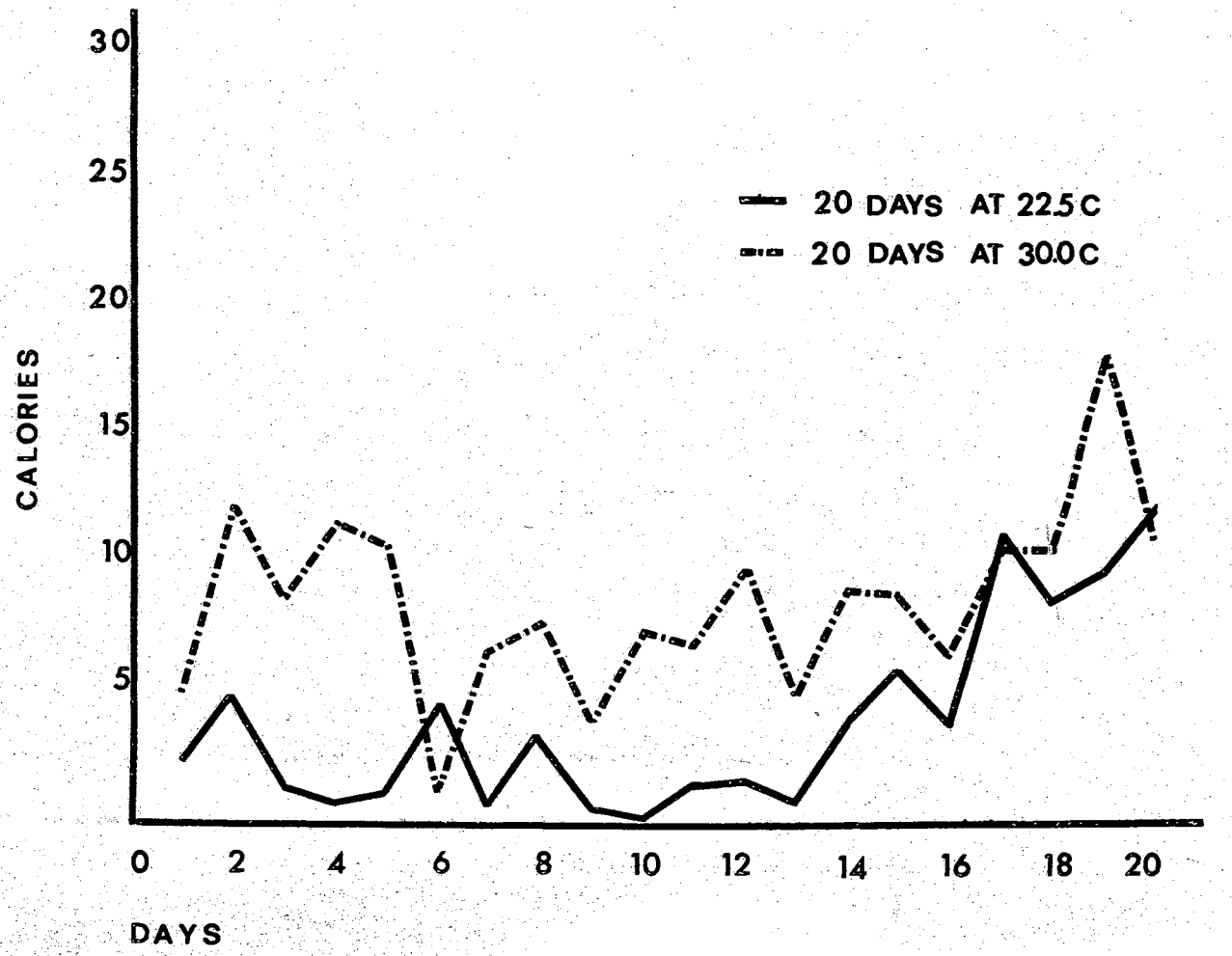

Fig. 6. Mean Daily Caloric Intake from Oil in Calories/100g Body Weight for 8 Kangaroo Rats in Experiment I

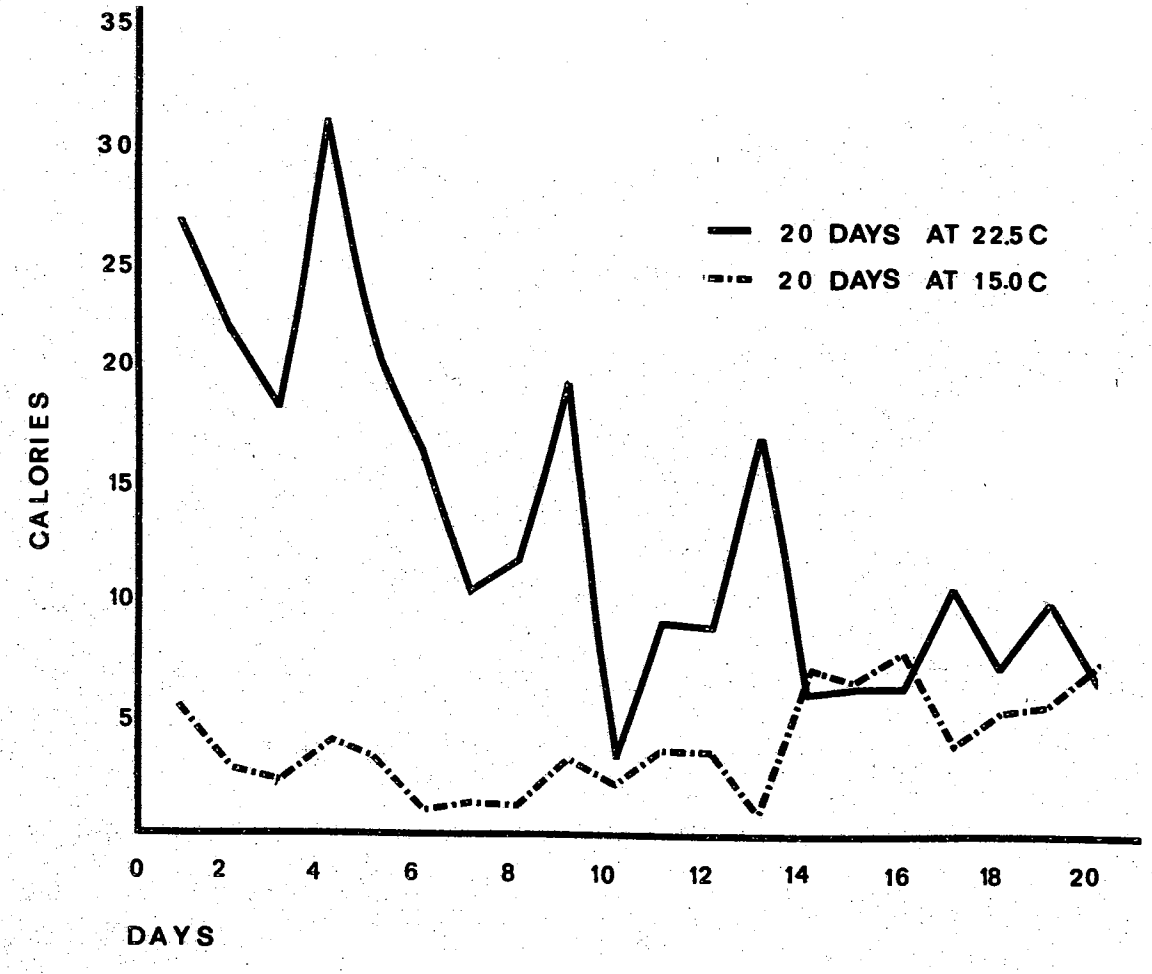

Fig. 7. Mean Daily Caloric Intake from Oil in Calories/100g Body Weight for 6 Kangaroo Rats in Experiment II

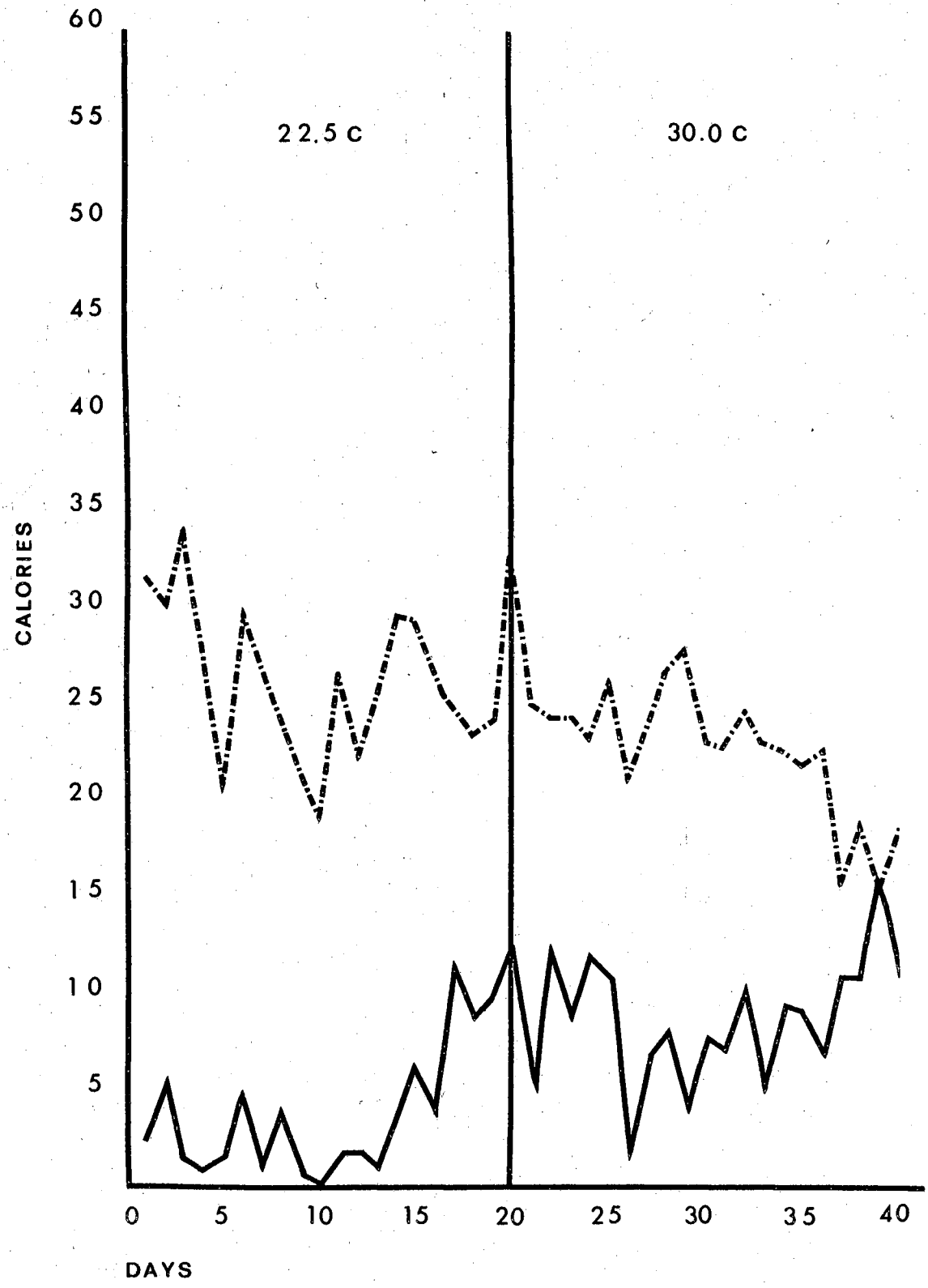

Fig. 8. Mean Daily Caloric Intake from Oil and Mash in Calories/100g Body Weight for 8 Kangaroo Rats in Experiment I

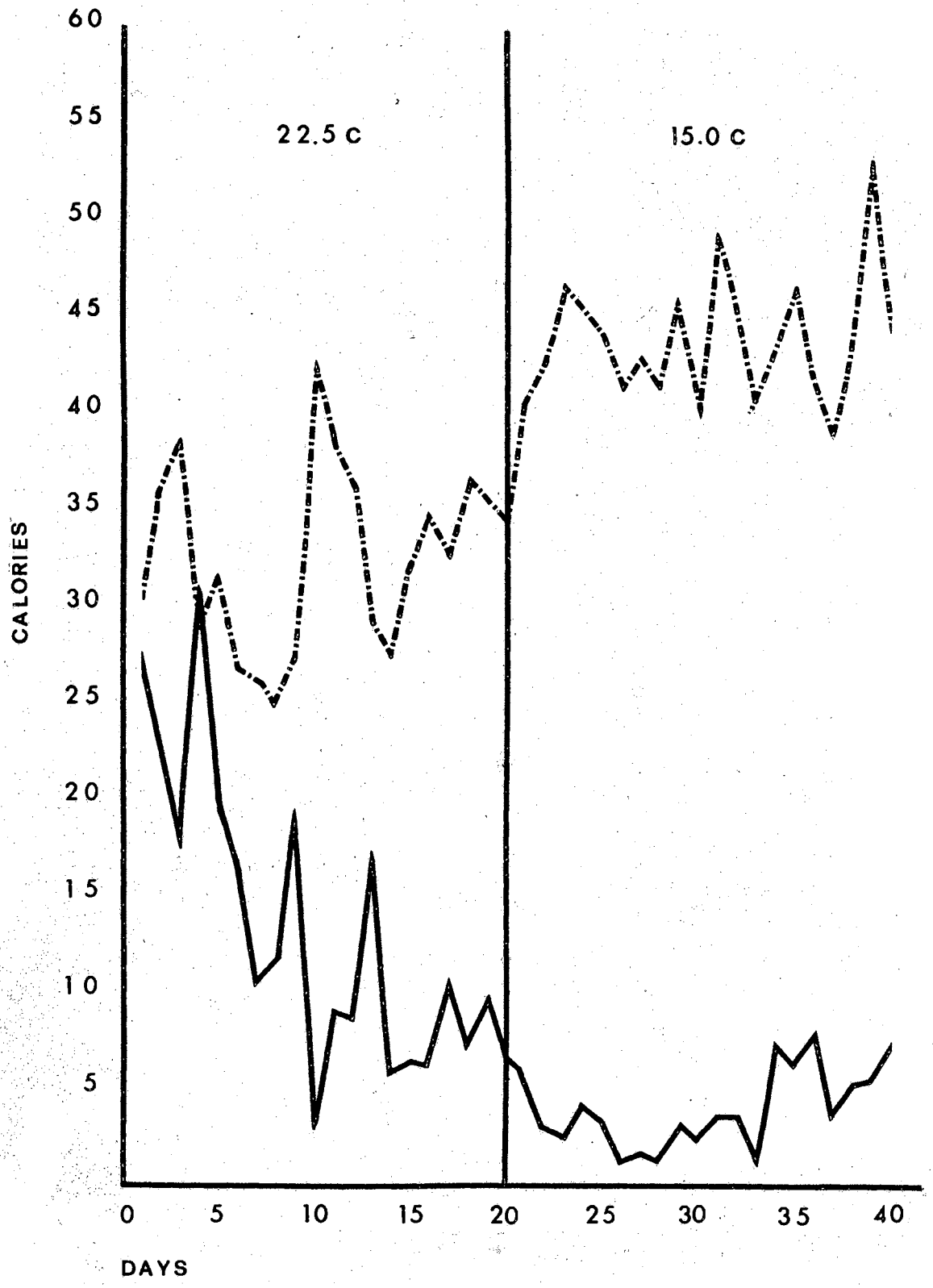

Fig. 9. Mean Daily Caloric Intakes from Oil and Mash in Calories/100g Body Weight for 6 Kangaroo Rats in Experiment II.

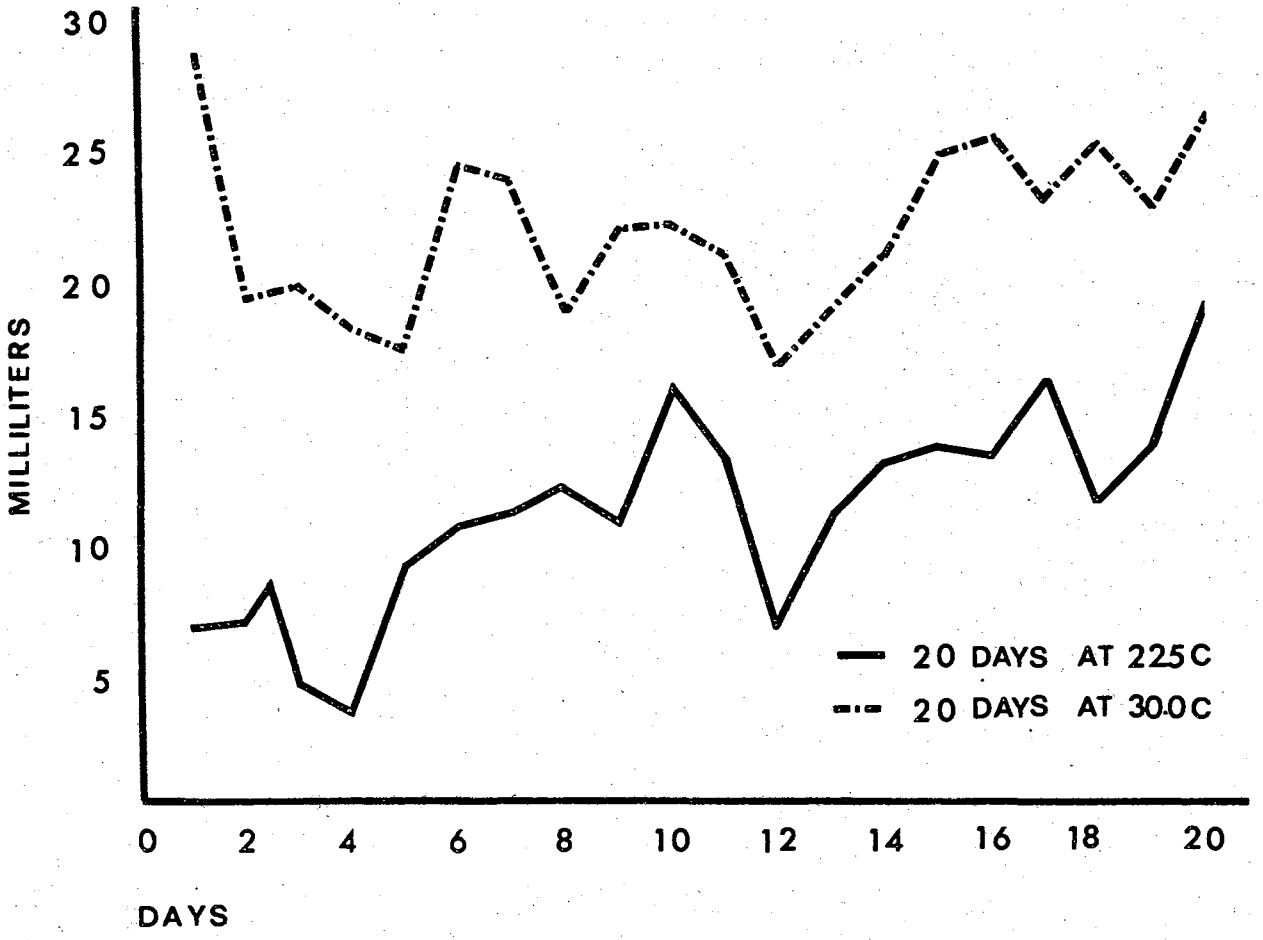

Fig. 10. Mean Daily Vitamin-Water Intakes in ml/100g Body Weight for 8 Kangaroo Rats in Experiment I

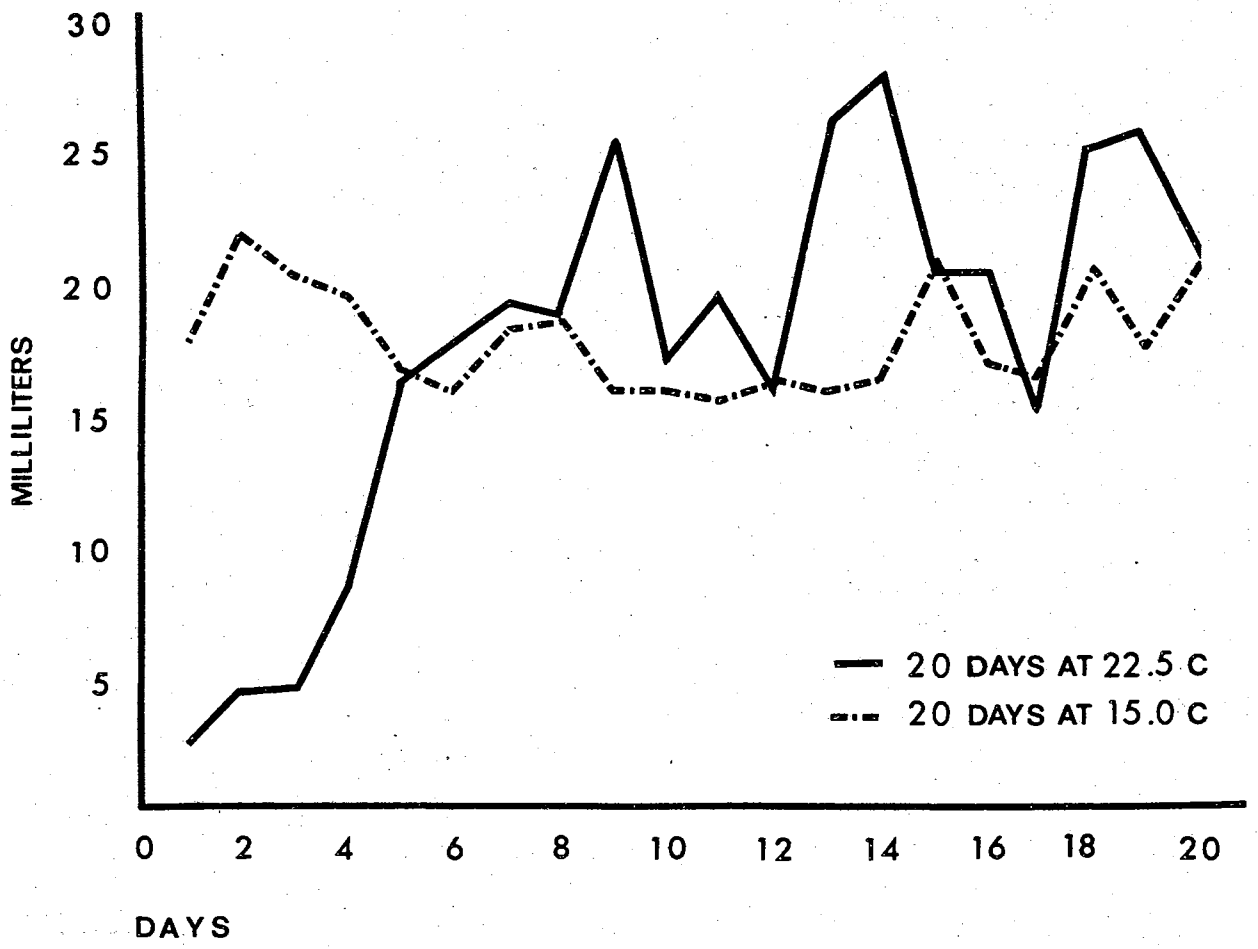

Fig. 11. Mean Daily Vitamin-Water Intakes in ml/100g Body Weight for 6 Kangaroo Rats in Experiment II.

first 20 days and increasing for the second 20 days (Fig. 12), but this is not related to the effects of temperature and the mean for both temperatures remained about the same (Table VI). The aminals remained at approximately the same mean body weight throughout both temperatures in Experiment II (Fig. 13).

Survival

In Experiment I, where 16 animals were tested and four deaths occurred, two of the deaths were due to not adjusting to the diet. The number of deaths was not significantly different at 30 C from that at 22.5 C (Table V). In Experiment II where fifteen animals were tested and seven deaths occurred, all of the deaths were due to inability to adjust caloric intake (Table V).

TABLE V

NUMBER OF DEATHS AT EACH TEMPERATURE SETTING DUE TO INABILITY TO ADJUST CALORIC INTAKE

	Experiment I	Experiment II
Temperature - 1	1	1
Temperature - 2	1	7

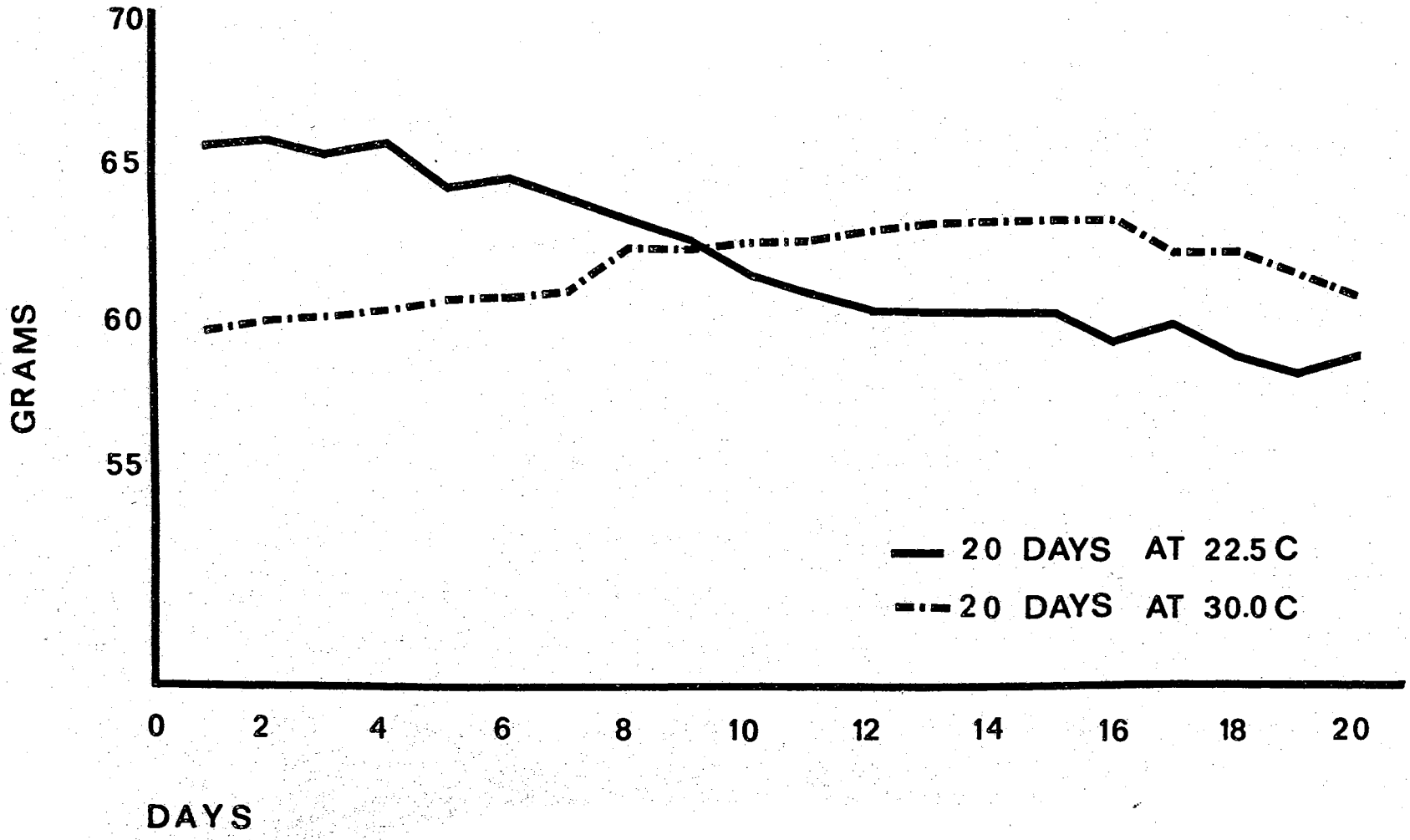

Fig. 12. Mean Daily Body Weight in Grams for 8 Kangaroo Rats in Experiment I.

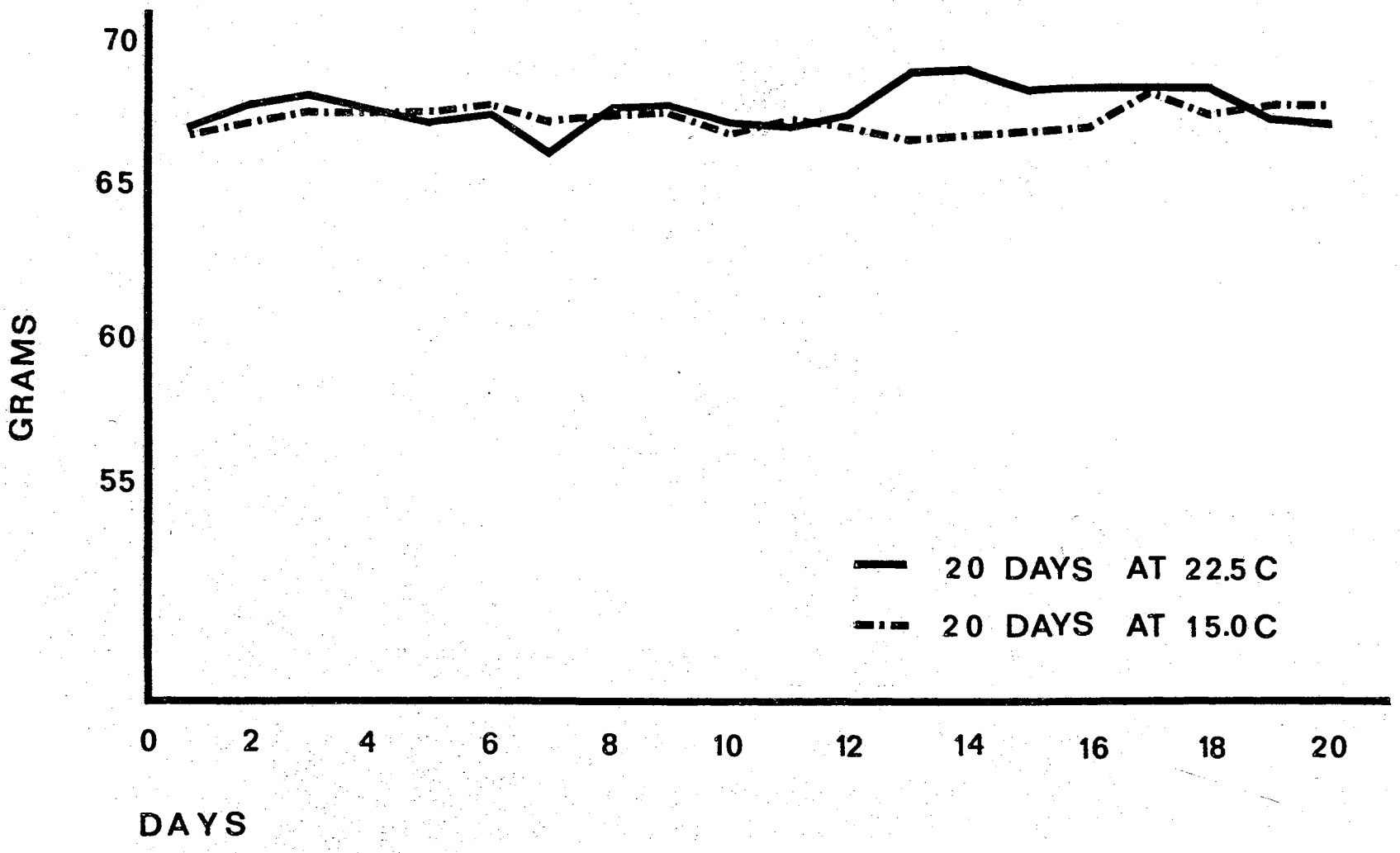

Fig. 13. Mean Daily Body Weight in Grams for 6 Kangaroo Rats in Experiment II

Summary of Means for Each Variable

In each experiment the average caloric intake from the diet at each temperature setting was nearly the same for both temperatures. In Experiment I, when the calories of mash decreased by 4.1 calories, the calories of oil increased by 4.5 calories. The average caloric intake from the diet differed by only .5 calories. The opposite adjustment occurred with the same overall result when temperature was decreased. In Experiment II, the calories from mash increased by 11.4 calories as oil intake decreased by 9.5 calories, resulting in the average caloric intake differing from the diet by only 2 calories.

TABLE VI

SUMMARY OF MEANS FOR EACH VARIABLE MEASURED

Experiment I	Calories from diet	Calories from mash	Calories from oil	Water intake	Body weight
Temperature - 1	30.6	26.5	4.1	10.8	62.0
Temperature - 2	31.1	22.4	8.6	21.8	61.7
Experiment II	**Total calories**	**Calories mash**	**Calories oil**	**Water intake**	**Body weight**
Temperature - 1	45.9	32.5	13.4	18.2	67.6
Temperature - 2	47.9	43.9	3.9	18.5	67.3

CHAPTER IV

DISCUSSION

Patterns of Caloric Adjustment

The results of the period at which this subspecies was maintained at 22.5 C were remarkably similar to that found by Warkentin, Warkentin and Ivy (1943) for normal rats at room temperature. The intake of calories from the diet was maintained, with the usual fluxuations, by the mash intake that was inversely related to the fat intake. There is well documented evidence of the alternate intakes of carbohydrate and fat (Warkentin, Warkentin, and Ivy, 1943; Lusk, 1928). Protein consumption, although not as directly related to fat intake, is usually in the opposite direction from fat because protein yields carbohydrate upon metabolism (Lusk, 1928). Since 87% of the mash used in this study was composed of protein or sugar, the inverse relationship of the mash and the oil intakes was as expected.

When temperature was changed the caloric intake from the diet remained relatively constant. This indicated that the intake at room temperature was adequate to meet the caloric requirements of the temperatures tested. The total caloric intake was maintained by regulating the source of calories to meet the different needs of each temperature.

Survival at low temperatures is associated with an increase in fat

and casein consumption when free choice is allowed. In this study, the subjects could choose only between supplemental oil or mash. If the supplemental oil was chosen it would cause a corresponding decrease in the intake of mash because of the carbohydrate present. This, in turn, would result in a decreased protein ingestion. Similarly, the choice of protein would decrease the intake of oil. At 15 C, the subjects that survived increased mash intake and decreased oil intake. This indicated that protein plays a greater role in survival at lower temperatures than previously thought.

With an increase in temperature the oil intake was adjusted immediately and followed the same pattern as at room temperature. The plot of mash intake at 30 C was superimposed on the plot of 22.5 C until day 13. After this the pattern of mash intake declines as oil intake increases. The change in mash intake at the increased temperature was not shown statistically (Appendix A) because the analysis compares only the average of the two temperatures. At the increased temperature the kangaroo rats also changed the source of calories. The oil was increased and the mash decreased to obtain the same caloric intake from the diet at 30 C as 22.5 C.

The patterns of caloric intakes were important. The animals had adjusted intakes to the 22.5 C temperature. If the intakes were adjusted at the changed temperature, the patterns should be the same as that at 22.5 C. The pattern of caloric intakes at 22.5 C and 30 C were different for the mash and diet. This means that the caloric intakes that were not adjusted immediately, continued to be adjusted throughout the test period. This is illustrated by the graphs (Figs. 2 and 4) and by the day temperature interaction in Appendix A.

The pattern for caloric intakes was the same at 22.5 C as 15 C for the oil, mash and diet. This indicated that the animals that survived had adjusted their intakes to meet the needs of that temperature. The adjustment to 15 C was more critical than to 30 C. If the kangaroo rat could not make a rapid adjustment, it died. Since only those individuals that survived were evaluated, the results show adjusted patterns of caloric intakes. This is shown both by the graphs (Figs. 3, 5, and 7) and by the lack of day temperature interaction in Appendix B.

Directional Changes in Water Intake

Whether water intake was adjusted or remained approximately the same was dependent upon the direction of the temperature change. This directional adjustment may facilitate maintenance of body temperature. Since reducing water intake below a certain level would not prevent heat loss, the animals may ingest water at a certain level unless greater quantities of water would prevent the elevation of body temperature.

The pattern of water ingestion was the same for 22.5 C and 30 C degrees, which suggested that water ingestion had been regulated to meet the water requirements of the increased temperature (Fig. 10, Appendix A). The pattern of water intakes were not the same at 22.5 C as 15 C, because the change in temperature did not require an immediate adjustment of intakes (Fig. 11, Appendix B).

Maintenance of Body Weight

Body weight did not change significantly due to temperatue changes in either experiment. This indicated that the mash and oil contained everything necessary for maintenance of body weight at each temperature.

Inability of Some Individuals to Survive

Some individuals, as expected, were not able to adjust to the experimental diet. Those animals that could not adjust died. Adjustment to a lower temperature is more critical than adjustment to a higher temperature within the range of values tested. Table VI illustrates the greater inability to adjust at 15 C than at 22.5 C. The inability to adjust intake may be due to the failure of a regulatory mechanism to function, as is most likely in the case of isolated deaths, or to differences in certain individual metabolisms. The animals that had a slightly higher metabolism than others in the test group at 15 C may require more calories to maintain life than they could possibly consume (Kleiber, 1961; Adolph, 1947). Kleiber (1961) suggests that survival of starving rats is related to metabolic rate.

Loss of body weight due to insufficient intake is apparent during starvation. This loss of weight has survival value under natural conditions. When no food is available to provide heat for the maintenance of body temperature, the tissues of the animal are katabolized to provide the heat necessary. This results in the weight loss.

The relative weight loss is more important than absolute weight loss since it can be used to compare animals of different weights. The percentage of the original weight lost is commonly used as a

measure of the relative weight loss in survival studies (Kleiber, 1961). In this study, all animals that could not adjust intake died at approximately the same percentage of the initial weight loss (30.5 ± 4.2).

CHAPTER V

CONCLUSIONS

Kangaroo rats of the subspecies Dipodomys ordii richardsoni maintained caloric intake from the diet at 15 C and at 30 C at the same level as when they were at an environmental temperature of 22.5 C. The sources of caloric intake was dependent on temperature. At the decreased temperature, calories from the oil were decreased and the calories from the mash increased to closely approximate the average caloric intake from the diet. The pattern was reversed when temperature was raised. The adjustment to 15 C occurred rapidly in all individuals that survived. The animals did not change mash intake immediately at 30 C, but required a period of adjustment.

Survival indicated that the need for protein was greater than the need for oil at a lowered temperature. It is suggested that future studies be performed to further investigate the inter-relationship of protein intake and the increased survival of animals at lower environmental temperatures.

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APPENDIX A

ANALYSIS OF VARIANCE IN EXPERIMENT I

Source of Variation	df	M. S. For Each Variable Measured				
		Calories from Oil	Calories from Mash	Calories from Diet	Water Ingested	Body Weight
Individuals	7	344.04	390.15	615.36	3465.62	955.65
Treatment	1	1688.86*	1363.04	17.44	9698.11*	6.69
Error a	7	230.04	303.71	195.91	1236.12	122.92
Days	19	2768.54**	104.01**	152.54	160.03	14.11**
Days X Temperature	19	52.50	131.21**	269.87**	55.61	52.92**
Error b	266	65.75	48.81	93.46	113.56	4.04

* = statistically significant at the 5% level

** = statistically significant at the 1% level

APPENDIX B

ANALYSIS OF VARIANCE IN EXPERIMENT II

Source of Variation	df	M. S. for Each Variable Measured				
		Calories from Oil	Calories from Mash	Calories from Diet	Water Ingested	Body Weight
Individuals	5	167.13	1320.19	1994.25	2622.06	1220.34
Treatment	1	5352.30**	7851.84**	238.73	6.43	6.27
Error a	5	37.40	45.74	43.80	254.41	203.97
Days	19	164.56**	126.09	223.66	144.61**	1.63
Days X Temperature	19	212.04**	80.63	229.82	209.70**	2.11
Error b	180	59.18	81.19	145.39	52.05	3.04

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